

tural sequence and degradation of nucleic acid have been noted¹¹⁻¹³, and our observation of the transformation of capsular type antigen of *S. aureus*¹⁴ reflected classical studies described by Avery¹⁵. Genetic mechanisms are probably involved in cellular injuries due to freezing and drying in addition to the unknown factor of maintenance

for a long time¹⁶. While biochemical investigations are required to elucidate this finding further, these observations already cast doubt on whether the capsular types of bacterial strains widely used are reliable, and emphasize the need to reexamine representative capsular type strains and reference antisera.

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On the interchromosomal connections in plants¹

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Summary. Using Giemsa C-banding technique, the existence of interchromosomal connections in well spread root tip metaphase plates in a variety of plant species has been demonstrated. Various types of interchromosomal connections are observed, involving satellite, telomeres, interstitial regions and in a few cases centromeres, too. The possible role of these interchromosomal connections in establishing the homologous and non-homologous association in the somatic chromosomes and maintaining the spatial relationship of the genetic apparatus is indicated. In the majority of the cases the connections are made up of constitutive heterochromatin.

The existence of interchromosomal connections during different stages of meiotic and mitotic cell cycle have been demonstrated in various cell types in animals using electron and light microscopic studies. These connections establish a supra-structure which govern the exact distribution of chromosomes during the cell cycle^{3,4}. In several plant species Wagenaar⁵ from his studies on telophase and early prophase in root tip cells suggested that chromosomes are attached end-to-end during interphase and these attachments form chain like interphase association. Almost similar type of associations among the chromosomes through heterochromatic interchromosomal connections have been reported in *Ornithogalum virens*⁶ and induced and/or revealed by 4-aminouracil in *Vicia faba*⁷.

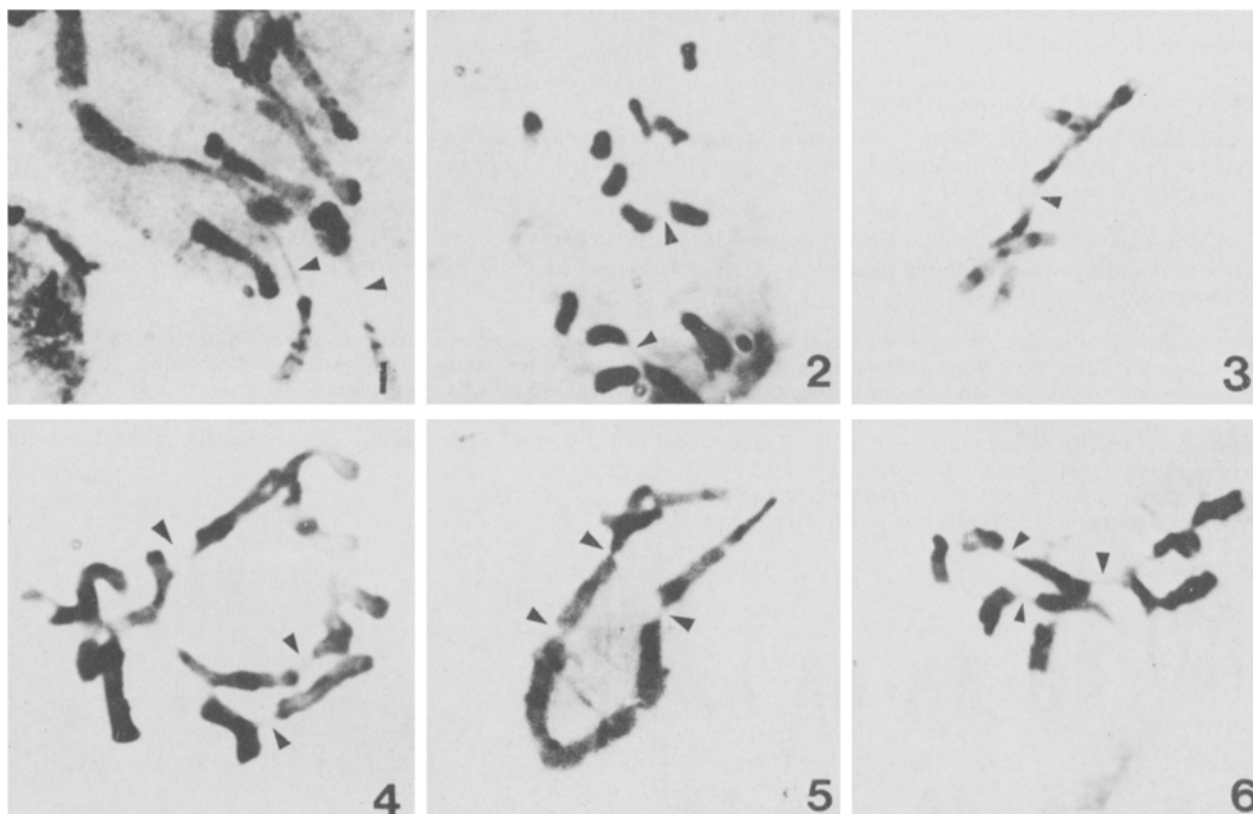
While working on the Giemsa C-banding patterns in various plant species in Leguminosae the authors noted very thin interchromosomal connections in well spread metaphase plates from root tips. The following types of chromatin connections could be delineated: a) Intersatellited connections, b) thin end-to-end connections among chromosomes, c) interstitial connections, d) centromere-to-centromere connections, e) centromere-to-telomere connections and a combination of above types.

The frequency of various types of connections varies in different plant types. The end-to-end connections are most frequently observed and centromere-to-centromere connections are rare. Approximately 80% of the connections observed are composed of heterochromatin. However, a smaller percentage is euchromatic too, as evidenced by C-banding technique. In order to ascertain the existence of the connections, control preparations were made to avoid

the possible chemical pretreatment effect (i.e. the effect of colchicine, etc.) and the resulting fixation artefact, if any. As such, the excised root tips were either directly fixed in Carnoy's fixative or fixed following a cold water treatment (near freezing temperature) for 24 h to facilitate chromosome separation, and processed as usual for C-banding⁸. In all such cases, connections were noted.

In view of these observations and a survey of a wide variety of plants with small and large sized chromosomes, it appears that these interchromosomal connections may be universally present either in a detectable or undetectable state. However, the frequency and ease with which these connections can be manifested, depend upon the technique of squashing pertaining to mechanical pressure applied, since such thin connections easily break. If one examines carefully a large number of cells in a monolayer group of cells (not the too much scattered and isolated cells) in a squash preparation made by applying uniform gentle pressure either on a macerated root tip tissue or the hydrolyzed small pieces of root tip followed by Giemsa C-band staining, the connections should be observed at least in a few cells. The monolayer small group of cells is especially suited for such an observation as it involves the minimum possible distortion of chromosomal material in a cell vis-à-vis revealing the chromosomal features.

The very fact that interchromosomal connections are more clearly observed after C-banding as compared to orcein or Feulgen staining suggests that C-banding may cause a swelling or dispersion of basic chromatin fiber to allow their visibility at the level of light microscope. In fact, such a situation was recorded by Schwarzsacher⁹ who suggested



Figures 1-6. Root tip metaphase cells showing various types of interchromosomal connections.

Figure 1. *Vicia lutea*. Figure 2. *Vicia disperma*. Figure 3. *Lathyrus sphaericus*. Figures 4 and 5. *Lathyrus sylvestris*. Figure 6. *Lathyrus odoratus*.

that C-banding treatment causes a dispersion of chromatin fiber and allows an additional swelling up to 3-times of the basic chromatin fiber, which in turn becomes more easily observable. A similar effect of a modified C-banding technique has been observed in animal chromosomes revealing the existence of interchromosomal connections which were not otherwise possible to locate after normal staining techniques¹⁰. In plants viz., *Ornithogalum virens*, the occurrence of interchromosomal connections has been clearly demonstrated using Giemsa C-banding technique. The fact that most of these connections involve specific chromosomes/chromosome segments and are clearly made up of distinct chromatin strands, sometimes several μm long, suggests the possibility of these interchromosomal connections to be of native occurrence rather than artefacts⁶. Heterochromatin is often described as being 'sticky', and fusion of constitutive heterochromatin is generally accepted and supported by extensive literature^{11,12}. There may be a possibility that a few of such interchromosomal connectives, particularly those involving closely associated chromosomes with very short connectives are due to heterochromatic stickiness to some extent but the relatively long and clear thread like connectives seem certainly of native nature. The present Giemsa C-banding study on interchromosomal connections in a wide range of plants has shown that most of the connections (approximately 80%) are composed of constitutive heterochromatin. Similar observations were recorded in *Ornithogalum virens* too, wherein nearly 90% of the connections were found to be of heterochromatic nature⁶.

It is likely that these interchromosomal connections maintain the spatial relationship of genetic apparatus throughout the division cycle^{4,13} and help to associate homologous

and non-homologous somatic chromosomes⁶. In eukaryotic systems, the genetic expression may not necessarily depend on the genes in a single chromosome or a segment¹⁴ and connections have been visualized to play a role in maintaining an interchromosomal system for coordinate functioning of the whole genetic apparatus¹⁵.

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